Synthesis of a 9-Acridinyl Nonapeptide Containing the DNA Recognizing Region of 434 Phage Repressor Protein

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Received November 28, 1995

A peptide-intercalator conjugate was synthesized by connecting acridine with a Gln-Gln-Ser-Ile-Glu-Gln-Leu-Glu-Asn sequence representing the DNA recognizing region of 434 phage repressor protein. This conjugate **7** binds to DNA in spite of its anionic character with the aid of the intercalator.

J. Heterocyclic Chem., **33**, 2043 (1996).

Introduction.

Intercalators interact with DNA reversibly through the intercalation between adjacent base pairs [1]. When positioned properly, a substituent on the intercalator molecule can be brought to the groove of DNA [1]. If this substituent is able to interact with DNA, the binding affinity of the intercalator would be increased. In fact, previous studies [2,3] suggested that the hydrogen bonding interaction of the terminal carboxamide group of intercalator with the adjacent guanine base took place. This kind of extra interaction is difficult to prove in most cases, however, unless a marked change in such properties as absorption is generated on the substituent upon its binding to DNA. Nevertheless, the binding constant of the intercalator for DNA should be altered by the presence of an extra interaction and this alteration may be taken as indirect evidence for the accurrence of such interactions.

Recent studies such as the single crystal X-ray diffraction analysis have shown the details of the interaction of DNA binding proteins with DNA [4]. Many workers have been studying the interaction with DNA of synthetic peptides deriving from the DNA binding proteins [5-10]. The model peptides studied were basic in nature. This character alone is favorable for the interaction of the peptides with polyanionic DNA. However, some of the DNA binding domains of proteins is totally acidic [4] and the intrinsic affinity of such domains for DNA should be low. Therefore the interaction of model peptides representing such a domain cannot be studied with polyanionic DNA. If the acidic peptides are connected to an intercalator molecule, the binding ability of the resulting conjugate will be enhanced to allow study of its interaction with DNA. With this consideration in mind, we connected a nonamer peptide containing the DNA recognizing region of 434 phage repressor protein with 9-acridinyl intercalator. Single crystal X-ray structural analysis of the complex of 434 phage repressor protein with oligonucleotide shows the direct interaction of nonameric peptide of α_3-helix moiety with 6-base pair of duplex DNA [11]. This peptide has three negative charges and the 9-acridinyl peptide is polyanionic as well.

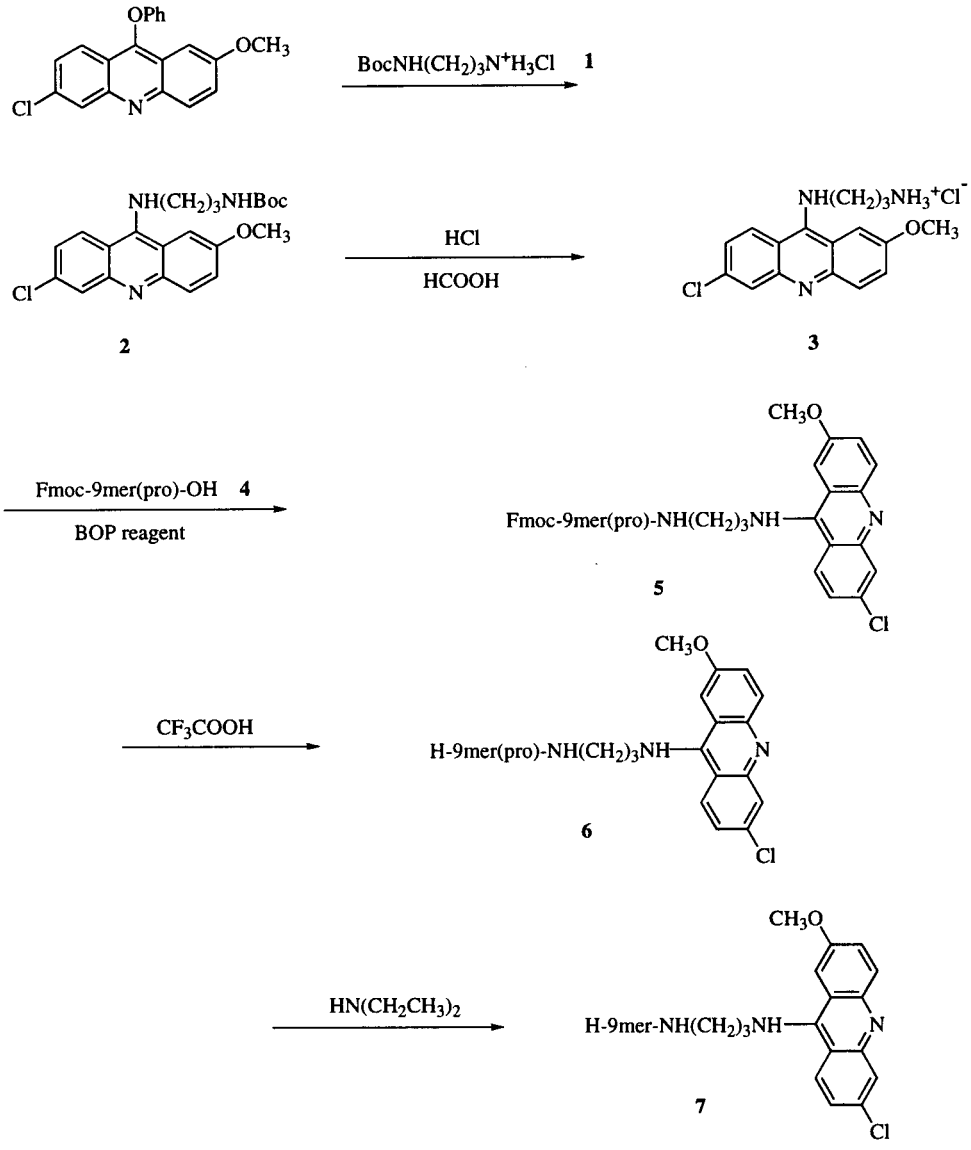
Results and Discussion.

9-Acridinyl peptide **6** was synthesized by connecting the amino moiety of 9-(3-aminopropylamino)acridine (**3**) with the carboxyl terminus of nonapeptide **4** in the following steps as outlined in Scheme I.

The previous paper showed the synthesis of **3** from the reaction of diaminopropane with phenoxyacridine in phenol [12]. However, this route gave the product only in impure form and in low yield. Hence, we first synthesized 3-*t*-butoxycarbonylaminopropylamine (**1**) and its subsequent reaction with phenoxyacridine afforded Boc(*t*-butoxylcarbonyl)-protected 9-aminopropylaminoacridine **2** as a precursor to 9-aminopropylaminoacridine. Since **3** tends to dimerize, it was generated immediately before the reaction with peptide by the reaction of Boc-protected 9-aminopropylaminoacridine **2** with formic acid and hydrochloric acid [13].

The nonameric peptide of the α_3-helix moiety of 434 repressor phage protein is H-Gln-Gln-Ser-Ile-Glu-Gln-Leu-Glu-Asn-OH [11] and was synthesized by the solid phase method using Fmoc (9-fluorenylmethyloxycarbonyl) chemistry with maximum protection of the side chain groups. Following detachment from the resin, the protected peptide was covalently attached to the terminal amino group of 9-(3-aminopropylamino)acridine (**3**) by BOP reagent (bis(2-oxo-3-oxazolidiny)phosphinic chloride). The 9-acridinyl peptide **7** was obtained after deprotection of the protecting groups of the side chains and the *N*-terminus of the peptide. The fraction soluble in 10% acetic acid aqueous solution of the crude precipitate gave

Scheme 1



9mer(pro): Gln-Gln-Ser(tBu)-Ile-Glu(OtBu)-Gln-Leu-Glu(OtBu)-Asn(Mbh)
9mer: Gln-Gln-Ser-Ile-Glu-Gln-Leu-Glu-Asn

almost pure product and it was further purified by reversed-phase hplc. We also synthesized the nonapeptide **8** for comparison.

Figure 1 shows the CD spectra of 9-acridinyl peptide **7** in buffered solution containing 10 or 80% of trifluoroethanol. The CD band at 222 nm increased with an increase of trifluoroethanol. Since the α-helix of peptides give rise on to absorption band at 222 nm, this result demonstrated that the α-helix was induced on the peptide **7** in trifluoroethanol. We attempted to measure the CD spectra of **7** in the presence of calf thymus DNA. However, the precipitates appeared after the titrations of DNA.

The absorption spectra of the acridine chromophore of compounds **2** and **7** show 40% and 24%, respectively, of hypochromic shifts with 6 nm of a bathochromic shift in the presence of calf thymus DNA, suggesting that **2** and **7** intercalate between adjacent base pairs of duplex DNA. It is interesting that even acidic compound **7** can bind to DNA, though the magnitude of binding affinity of **7** is smaller than that of **2**. The ability of **7** to bind with the recognized oligonucleotide was evaluated from changes in the helix-coil transition temperature of DNA (T_m). The recognized oligonucleotide is the duplex of d(AATTCTACAAGAAAGTTTGTTG) and d(AATTCAACAAACT-

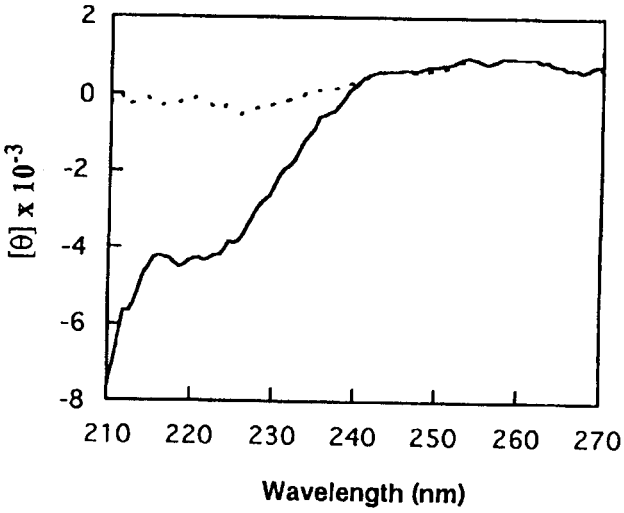


Figure 1. CD spectra of 9-acridinyl peptide **7** in 10 m*M* tris(hydroxymethyl)aminomethane hydrochloride buffer and 1 m*M* sodium chloride (*p*H 7.8) containing 10 (broken line) or 80% (solid line) of trifluoroethanol.

TTCTTGTAG). The difference in the T_m for oligonucleotide with and without **7** (ΔT_m = 2.0°), which is a measure of the binding affinity, indicated that **7** stabilized the oligonucleotide having the recognized sequence. However, the acridine part **2** also stabilized this oligonucleotide to the same extent (ΔT_m = 2.0°), whereas peptide **8** showed no effect on T_m.

In conclusion, owing to the intercalating interaction, the totally anionic peptide could bind with DNA. In other words, an intercalator can be used as a vector for a peptide to DNA.

EXPERIMENTAL

The ^{1}H-nmr spectra were obtained on a Hitachi R-24B and Jeol GSX-400 operating at 60 and 400 MHz, respectively, with tetramethylsilane as an internal standard. The ir spectra were recorded with a JASCO IR-800 instrument. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected.

Calf thymus DNA was purchased from Sigma Chemical Co. and purified as described previously [14,15]. The oligonucleotides, d(AATTCTACAAGAAAGTTTGTTG) and d(AATTCAACAAACTTTCTTGTAG) were synthesized on a DNA synthesizer (Applied Biosystems, PCR-MATE EP) by using cyanoethylphosphroamidite chemistry. *N*-(*t*-Butoxycarbonyl)propane-1,3-diamine (**1**) [16] and 6-chloro-2-methoxy-9-phenoxylacridine [17] were prepared according to the known method. Protected amino acids were purchased from Applied Biosystems. Standard three-letter notations are used for L-amino acids. Electronic absorption spectra were determined on a Hitachi U-3210 UV-visible spectrophotometer equipped with a temperature controller Hitachi SPR-10. Thermal denaturation experiments were conducted in 10 m*M* 2-(*N*-morpholino)ethansulfonic acid and 10.0 μ*M* ethylenediaminetetraacetic aicd (*p*H 5.9) and 0.1 *M* sodium chloride at a ratio of 2 moles of ligand per mole of DNA-ds and the DNA concentration was set to 0.5 μ*M*. A heating rate was set at 0.33°/minute. The ΔT_m is defined as the difference in T_m of DNA in the presence and absence of ligand. Circular dichroism spectra were recorded at 230-700 nm on a Jasco-J 500 spectropolarimeter at 25°. The hplc was run on a Hitachi L-6200 chromatograph with a dual plunger pump on a TSK GEL C18-4PW column (8 mm i.d. x 15 cm, Tosoh). The eluent, consisting of 0.1% trifluoroacetic acid (TFA) (A) and 0.1% TFA in 70% acetonitrile (B), was run in a linear gradient of 3-97% in 40 minutes (condition 1) or 20-100% in 25 minutes (condition 2) at a flow rate of 1.0 ml/minute.

N-[9'-(6'-Chloro-2'-methoxyacridinyl)]-*N'*-*t*-butoxycarbonylpropane-1,3-diamine (**2**).

6-Chloro-2-methoxy-9-phenoxylacridine (0.92 g, 2.8 mmoles) and *N*-(*t*-butoxycarbonyl)propane-1,3-diamine (**1**) (0.58 g, 2.8 mmoles) were dissolved in phenol (20 g) and the solution was heated at 100° for 5 hours. The solution was allowed to cool and poured into ether (200 ml). The residue obtained was chromatographed over silica gel using chloroform, methanol, and diethylamine (9:1:1) as an eluent and was crystallized from methanol to yield 0.22 mg (19%) of (**2**) as a yellow solid, mp 206-207°; ^{1}H-nmr (400 MHz, DMSO-d_6): δ 1.30 (s, 9H), 1.80 (m, 2H), 3.00 (m, 2H), 3.70 (m, 2H), 3.90 (m, 3H), 7.20-8.40 (m, 6H).

Anal. Calcd. for $C_{22}H_{27}N_3O_3Cl_2 \cdot H_2O$: C, 56.17; H, 6.17; N, 8.94. Found: C, 56.47; H, 6.50; N, 9.00.

9-(3-Aminopropylamino)acridine (**3**).

To a solution of **2** (0.20 g, 0.48 mmole) is added at room temperature 0.1 *M* HCl/HCOOH (5.8 ml, 0.58 mmole). The mixture was stirred at room temperature for 1 hour and then poured into ether (500 ml). The solid obtained was dried under reduced pressure and used in the following reaction: TLC R_f = 0 (chloroform:methanol = 8:1), ninhydrin test +, ir (potassium bromide): the peak of Fmoc disappeared.

Fmoc-Gln-Gln-Ser(*t*-Bu)-Ile-Glu(*O*-*t*-Bu)-Gln-Leu-Glu-(*O*-*t*-Bu)Asn(Mbh)-OH (**4**).

This peptide was synthesized on a peptide synthesizer (Applied Biosystems, 431A) by using Fmoc chemistry and a Sasrin Resin (super acid sensitive resin, 1.06 mmoles/g) from Kokusan Chemical Works. Starting with 236 mg of Sasrin Resin, 421 mg of the peptide-immobilized resin were obtained. A portion (100 mg) of this sample was suspended in 1% TFA/dichloromethane (10 ml) and stirred at room temperature for 15 minutes. Pyridine (50 μl) was added and the resin was filtered and washed with dichloromethane several times. These procedures were repeated three times. The combined solution was evaporated *in vacuo* and added to ether (50 ml). The precipitate formed was collected, washed with water and methanol and dried.

H-Gln-Gln-Ser-Ile-Glu-Gln-Leu-Glu-Asn-NH$(CH_2)_3$-(9-Acr) (**7**).

The side-chain protected peptide **4** (122 mg, 80 μmoles) and 9-(3-aminopropylamino)acridine (**3**) (68 mg, 160 μmoles) were dissolved in anhydrous (dimethyl formamide) (3 ml) and DMSO (2 ml). Trieth-ylamine (0.1 ml, 720 μmoles) and POP reagent (110 mg, 250 μmoles) were added and the reaction mixture stirred at 0° for 6 hours. The solvent was removed and the residue was poured into methanol. The solid obtained was

washed with ether and dried under reduced pressure to yield 96 mg (65%) of **5** as a yellow solid.

The solid was dissolved in TFA (9.5 ml) containing ethylene glycol (0.1 ml) and anisole (0.3 ml) and stirred at room temperature for 1.5 hours. The solvent was removed under reduced pressure and the residue was diluted with ether. The precipitate formed was collected. After washing it with 0.1% acetic acid aqueous solution, **6** was obtained as a single peak on hplc. The retention time for **6** was 20.0 minutes under conditions 2, yield 23 mg (22%) of **6** as a yellow solid: ninhydrin test.

Peptide **6** (5 mg, 3.1 μmoles) was dissolved in ~10% dimethylamine/DMF (3 ml) and stirred at room temperature for 1 hour. The solvent was removed and the residue was washed with hexane and ethyl acetate. Compound **7** was further purified by hplc to homogeneity: the retention time for **7** was 21.7 minutes under conditions 2, yield 3.9 mg (90%) of **7** as a yellow solid: ninhydrin test +, ir (potassium bromide) the peak of Fmoc disappeared.

H-Gln-Gln-Ser-Ile-Glu-Gln-Leu-Glu-Asn-OH (**8**).

This peptide was synthesized on a peptide synthesizer (Applied Biosystems, 431A) by using Fmoc chemistry. The crude material was further purified by hplc. The retention time for **8** was 27.4 minutes under conditions 1. The peptide structure was determined by amino acid analysis and peptide sequencing; AAA expected, $Asn_1Gln_3Glu_2Ile_1Leu_1Ser_1$: Found, $Asp_{0.9}$-$Glu_{5.3}Ile_{1.0}Leu_{1.1}Ser_{0.7}$. The decomposition of Ser was not corrected for.

Acknowledgments.

The authors are grateful to Dr. H. Kondo (Kyushu Institute of Technology) for reading the manuscript. The present study was partially supported by the Nissan Science Foundation (to S.T.) and Saneyoshi Scholarship Foundation (to S.T.) and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES AND NOTES

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[1] W. D. Wilson and R. L. Jones, Intercalator Chemistry, M. S. Whittingham and A. J. Jacobson, eds, Academic Press, New York, 1982, p 445.

[2] B. Gaugain, J. Markovits, J.-B. Le Pecq and B. P. Roques, *Biochemistry*, **20**, 3035 (1981).

[3] J. Markovits, B. Gaugain, J. Barbet, B. P. Roques and J.-B. Le Pecq, *Biochemistry*, **20**, 3042 (1981).

[4] A. Goldman and D. L. Ollis, Nucleic Acids in Chemistry and Biology, G. M. Blackburn and M. J. Gait, eds, IRL Press, New York, 1990, p 337.

[5] M. G. Oakley and P. B. Dervan, *Science*, **248**, 847 (1990).

[6] R. V. Talanian, C. J. McKnight and R. S. Kim, *Science*, **249**, 769 (1990).

[7] R. V. Talanian, C. J. McKnight, R. Rutkowski and R. S. Kim, *Biochemistry*, **31**, 6871 (1992).

[8] B. Cuenoud and A. Schepartz, *Science*, **259**, 510 (1993).

[9] C. R. Palmer, L. S. Sloan, J. C. Adrian, Jr., B. Cuenoud, D. N. Paolella and A. Schepartz, *J. Am. Chem. Soc.*, **117**, 8899 (1995).

[10] T. Morii, M. Simomura, S. Morimoto and I. Saito, *J. Am. Chem. Soc.*, **115**, 1150 (1993).

[11] J. E. Anderson, *Nature*, **183**, 553 (1987).

[12] E. S. Canellakis, Y. H. Shaw, W. E. Hanners and R. A. Schwartz, *Biochim. Biophys. Acta*, **418**, 277 (1976).

[13] The previous paper [12] suggested 9-aminopropylacridine underwent spontaneous dimerization.

[14] W. D. Wilson, Y. H. Wang, C. R. Krishnamoorthy and J. C. Smith, *Biochemistry*, **24**, 3991 (1985).

[15] W. D. Wilson, C. R. Krishnamoorthy, Y.-H. Wang and J. C. Smith, *Biopolymers*, **24**, 1941 (1985).

[16] J. B. Hansen, M. C. Nielsen, U. Ehrbar and O. Buchardt, *Synthesis*, 404 (1982).

[17] D. J. Dupre and F. A. Robinson, *J. Chem. Soc.*, 549 (1945).